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STUDIES ON EFFICACY OF ENTOMOPATHOGENIC FUNGI *Metarhizium anisopliae* (METCHNIKOFF) SOROKIN AGAINST *Nilaparvata lugens* (Stål)

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ABSTRACT

Experiments were conducted to evaluate the three Metarhizium anisopliae strains were collected from the various institutes. Among the three strains, Metarhizium (M1) strain (NBAIR) showed a higher percentage of mortality against N. lugens (76.67%) under in vitro conditions. Four concentration of each of the strain were used along with untreated control to determine the LC_{50} and LT_{50} for the Metarhizium strains. The least LT_{50} value of 4.4 days was registered in M1 strain with the spore concentration of 1 X 10^8 and the LC_{50} value was 3.4 X 10^4 respectively. Metarhizium (M1) strains altered the feeding behavior and nymphal emergence of N. lugens under screen house condition.

KEYWORDS: Metarhizium anisopliae, Nilaparvata lugens, Rice, Concentration Mortality, Time Mortality

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Original Article

INTRODUCTION

Rice Brown plant hopper *Nilaparvata lugens* (Stal) considered as one of the major pests in the past in many Asian countries, has assumed important pest status and becomes a major threat to rice production in tropical and subtropical Asia (Khan *et al*., 1988). It has monophagous insect pests and infect rice plants from seedling stage to maturity stage causing considerable yield losses (Kiritani, 1979). It was estimated that the yield reduction due to brown plant hopper infestation was 48-57% (Murugesan and Chelliah, 1983) and 60% respectively (Panda and Khush, 1995). Several insecticides that are mostly effective as foliar sprays have been identified for control of rice brown plant hopper but some of the earlier effective as foliar insecticides are now reported to cause resurgence, environmental pollution, contamination of food materials, toxicity to natural enemies and hazards to human beings (Endo *et al*., 1993; Xiao *et al*., 1994 and Natarajan and Ramaraju 1997). These problems have necessitated the scientists to look for the alternative avenues and in this context, microbial agents play a leading role as a component of the integrated pest management.

Limited information is available on such studies, especially in rice. The talc-based formulation containing beneficial microbes was found to be effective and cheaper for pests and diseases in different crops (Saravanakumar *et al*., 2007; Rajendran *et al.,* 2007; Radjacommare *et al*., 2002). Hence, the present study was carried out with the objectives of (i) Collection of effective *Metarhizium* and in vitro bioassay against the rice brown plat hopper and (ii) Trying the talc-based powder formulation containing *Metarhizium* strain and evaluating its efficacy against brown plant hopper under screen house conditions.

MATERIALS AND METHODS

Source of the Fungi

Pure cultures of the entomopathogenic fungi, *M. anisopliae* were obtained from National Bureau of Agricultural Important Insects (NBAIR) Bangalore, Sugarcane Breeding Institute (SBI), Tamil Nadu Agricultural University (TNAU) Coimbatore. The cultures were routinely maintained in artificial media (PDA) utilizing standard techniques Aguda *et al.*, (1987), (Table 1).

Mass Rearing of Brown Plant Hopper

Plant material and brown plant hopper was mass rearing Rice cultivar TN1 from Paddy Breeding Station, TNAU, Coimbatore, India was used for evaluating the efficacy of entomopathogenic fungal pathogens against brown plat hopper infection in rice plants. The method outlined by Medrano and Heinrichs (1985) was followed in mass culturing of BPH. Germinated rice seeds were sown in 40 x 30x5 cm trays and after 15 days, the seedlings were transplanted into earthen pots (12 cm dia) and kept partially immersed in plastic tray containing water. These trays were kept inside the insect rearing cages (3 x 2.5 x 2.75 m) with galvanized iron wire netting. Gravid females were collected from the field and released on individual potted plants kept inside the rearing cages for oviposition. Nymphs emerged a week after oviposition and the damaged plants due to feeding of BPH were periodically replaced with fresh potted plants. The plants were made free of spiders, mirid bugs and ants for effective culturing of BPH

Conidial Suspension Preparation

Mycelial discs of *M. anisopliae* were inoculated in SDY broth supplemented with 1% yeast extract and incubated at 26^0C for 48 h with shaking at 180 rev min-1. The fungal spores were harvested in 25 ml of sterilized distilled water (SDW) containing 0.05% Tween 20 (Polyoxyethylene sorbitan monolaurate) and the spore count of this stock suspension was estimated with an improved Neubaur haemocytometer. The spore concentration of the isolates was adjusted to 10^2, 10^4, 10^6, and 10^8 spores ml-1 for *N. lugens* bioassay. The pathogens was re isolated from the treated dead larvae of *N. lugens* were used for further experiments.

Pathogenicity on Brown Plant Hopper

For the pathogenicity test, conidial suspension ($1x10^2$ to $1x10^8$) was used and Tween 80 (0.1%) was used as a sticking agent. Paddy stem cuttings (disinfected with 0.5% sodium hypochlorite) were dipped in this suspension for few seconds. Five ml of spore suspension was used for treating the stem cuttings. The stem cuttings was (disinfected with 0.5% sodium hypochlorite) treated with Tween 80 (0.1%) prepared in sterile distilled water was used as a control. After treatment, each stem cutting (disinfected with 0.5% sodium hypochlorite) was kept in separate test tubes containing 30 ml of sterile distilled water and ten BPH nymphs were released in each test tube and incubated at room temperature. The dead brown plant hopper nymphs and adults were placed in a sterile petri plate containing a moist cotton swab to allow mycelial growth over the cadaver. The mortality was recorded upto 10 days. Based on these results, best isolates were selected for further studies Sivasundaram *et al.*, (2008).

BIOASSAY *IN VITRO*

Bioassay of Entomopathogenic Fungal Isolates against BPH under i*n Vitro* Conditions

For the bioassay, four different concentrations viz., $1x10^2$, $1x10^4$, $1x10^6$ and $1x10^8$ conidia ml-1 were used. Tween

80 (0.1%) was used as a sticking agent and paddy stem cuttings (disinfected with 0.5% sodium hypochlorite) were dipped in this suspension for few seconds. Five ml of spore suspension was used for treating the stem cuttings. The stem cuttings (disinfected with 0.5% sodium hypochlorite) treated with Tween 80 (0.1%) prepared in sterile distilled water was used as a control. After treatment, each stem cutting (disinfected with 0.5% sodium hypochlorite) kept in separate test tubes containing 30 ml of sterile distilled water and ten BPH nymphs were released in each test tube. These tubes were then kept inside the BOD incubator at 25 ± 1^0 C and the nymphal mortality was recorded at 24 h interval until ten days of treatment. The percentage nymphal mortality due to mycosis was calculated. The results of the assay were subjected to probit analysis and the median lethal time (LT_{50}) and median lethal concentration (LC_{50}) for the virulent isolate were calculated.

Preparation of Talc-Based Formulation for Entomopathogenic Fungal Strains

Entomopathogenic fungal strains were multiplied in the molasses yeast medium (30 g molasses, 5 g yeast, and 1 l water). After multiplication, the broth containing 13x 10^7 c.f.u./ml in the flask was mixed with talc at 1:2 ratio (500 ml:1 kg). To the mixture, 5 g of CMC was added as sticker and dried in shade for 72 h, powdered and stored in polypropylene bags (Jeyarajan *et al.,* 1994). The population of entomopathogenic fungal strains during application was 1.1 x 10^8 for *Metarhizium* anisopliae (M1) strain.

Testing the Talc Based Bioformulations of Entomopathogenic Fungal Pathogens against BPH under Screen House Conditions

Second instar nymphs of *N. lugens* were bioassayed for their susceptibility to fungal pathogens. Ten nymphs were carefully collected from the cage by using aspirator, starved for 5 h and released on the treated plants covered with Mylar film on 30 days after planting. The talc based product of entomopathogenic fungal strains *M. anisopliae* (M1) (1×10^8) and *Metarhizium* (Kothari Pvt Ltd) for check (1×10^8) @ 20 g/l was mixed with water and allowed to settle for 1 h, and the supernatant solution was sprayed in BPH released treated plants using a hand atomizer. Three replicates of ten nymphs were used in each case and 0.03 % Dimethoate served as chemical check. Sterilized distilled water with 0.05 percent Tween 20 served as control. Three lots of plants sprayed with 10 ml of sterilized distilled water with 0.05 percent Tween 20 served as healthy control.

Effect of Entomopathogenic Fungal Pathogens on Feeding Activity of BPH

The modified method of Pathak and Heinrichs (1982) to capture the honeydew droplets was followed. Whatman No.1 circular filter paper was impregnated twice with bromocresol green solution (2 mg ml^{-1} ethanol) and it was allowed to dry for 1 h and then retreated with the solution till the filter paper turned to orange.

Forty days old TN1 potted plants of different treatments were used. A plastic dish was supported around each seedling over which the filter paper discs treated with bromocresol green were placed and the set up was covered with Mylar film which was covered with muslin cloth at the top. Five gravid females starved for 5 h were transferred to potted plants through the Mylar film opening at the top. The set up was kept undisturbed for 24 h and immediately upon contact with honeydew, blue spots appeared on the treated filter paper. As the concentration of honeydew increased the spot turned white in the centre with blue edges where the honeydew concentration was less. The margins of spots were drawn on a tracing paper and then the tracing paper was placed over a graph paper and the number of squares occupied by the spot was counted. Feeding activity of BPH was expressed as mm^2.

Effect of Entomopathogenic Fungal Pathogens on Nymphal Emergence of BPH

Ten second instar BPH nymphs were transferred to tubular pots containing rice seedlings by using aspirator. Each pot was confined with the mylar film covered with muslin cloth at the top. At the sex differentiation stage (fifth instar) only one gravid female was allowed to feed per pot and others were removed. Gravid females were allowed to oviposit till the end of life cycle. Number of nymphs emerged were counted daily up to 20 days from the period of oviposition Sivasundaram *et al.*, (2008).

Statistical Analysis

The data were statistically analyzed using IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez 1984). Prior to statistical analysis of variance (ANOVA), the percentage values were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels (P\0.05 and P\0.01) and means were compared by Duncan's Multiple Range Test (DMRT).

Log Dose Probit Analysis

Bioassay data from three replications of four concentrations were pooled and subjected to Probit analysis using software SPSS package for computing dose-mortality regression. The control mortality was corrected as per the Finney's method (Finney 1971).

RESULTS AND DISCUSSIONS

Collection of Entomopathogenic Fungal Pathogens and the Pathogenicity Test

Entomopathogenic fungal pathogens were collected from various institutes of India. From the institutes, 3 strains were collected and the conidial structures were observed by stereo zoom microscope. Differential percentage mortality was observed in *N.lugens* with respect to entomopathogenic fungi and their isolates. All the 3 isolates of *Metarhizium* were found to be pathogenic to *N. lugens* (Table 1). The percentage mortality ranged from 46.67 to 76.67. The M1 (NBAIR) isolate recorded a maximum mortality of 76.67%. No larval mortality was observed in the control. The pathogenicity study of Yasodha and Narayanasamy, (2004) reported that *Fusarium moniliformae* isolated from Annamalai nagar caused maximum mortality of 61.6 per cent which was higher than maximum mortality caused by *Mucor hiemalis* against both yellow stem borer and leaffolders under *in vitro* conditions.

Table 1: Collection of *Metarhizium* Strains from Various Institutes

S.No	Strains	Location	Pathogenicity %*
1.	NBAII (M1)	Bangalore	76.67 (61.14)[a]
2.	TNAU (M2)	Tamil Nadu	63.33 (52.74)[b]
3.	SBI (M3)	Tamil Nadu	46.67 (43.09)c
4.	Control		0.00 (0.36)d

*** NBAIR:** National Bureau of Agricultural Insects Resource
***TNAU:** Tamil Nadu Agricultural University
***SBI**: Sugarcane Breeding Institute

Values are mean of three replications Figures in parentheses represent arcsine transformation Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05

Concentration Mortaliy and Time Mortality Response of *Metarhizium* Strains against *N. Lugens* under *In Vitro* Conditions

Mortality was correlated with the concentration of conidial suspension. The studies indicate that the nymphal mortality was low. The least nymphal mortality was observed in the 1×10^2 (30.00 %) (Table 2). M1 isolate with concentration of $1x10^8$ registered a low LT_{50} value of 4.62 days followed by 1×10^6 (5.76 days). The maximum LT_{50} value was noted in 1×10^2 concentration (12.16 days) (Table 3). No nymphal mortality was observed in the control (Table 2). The LC_{50} of *Metarhizium* strain (M1) against *N. lugens* was 1.54×10^4 respectively. This result shows that *Metarhizium* (M1) strain is highly effective against *N. lugens.*

Table 2: Concentration mortality response of *Metarhizium* (M1, NBAIR) against BPH

Strain and Conidial Concentrations	Per Cent Mortality up to 10 Days	LC_{50} (Spores/ml)	Fiducial Limit (95%)		Slope (± S.E.)
			Lower	Upper	
Metarhizium (M1)					
1×10^2	30.000 (33.21)[d]				
1×10^4	46.670 (43.10)[c]				
1×10^6	66.670 (54.74)[b]				
1×10^8	76.670 (61.13)[a]	1.54×10^4	0.07	35.79	0.19 (0.12)
Control	0.00 (0.36)[e]				

Values are mean of three replications.

Figures in parentheses represent arcsine transformed values.

Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 3: Time mortality response of *Metarhizium* (M1, NBAIR) against BPH

Strain and Conidial Concentrations	LC_{50} (Spores/ml)	Fiducial Limit (95%)		Slope (± S.E.)
		Lower	Upper	
Metarhizium (M1)				
1×10^2	12.16	9.36	85.80	3.44 (1.38)
1×10^4	9.10	7.80	13.17	3.55 (0.91)
1×10^6	5.76	5.07	6.71	3.54 (0.72)
1×10^8	4.62	3.89	5.27	3.38 (0.70)
Control	0.0	-	-	-

Values are mean of three replications.

Figures in parentheses represent arcsine transformed values.

Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 4: Efficacy of Talc Based Bioformulation of *Metarhizium* Strains against Brown Plant Hopper (BPH) under Screen House Condition

S. No	Treatments	BPH Mortality (%)
1.	*Metarhizium*strain (M1,NBAII) 20g/lit of water	71.0 (57.44) [b]
2.	*Metarhizium* talc based (Commercial formulation of Kothari Pvt Ltd, for check) 20 g/lit of water	60.0 (50.78) [c]
3.	Chemical (chlorpyriphos, for check) 0.04% foliar spray	83.0 (65.70) [a]
4.	Control inoculated	0.0 (0.36) [d]

Values are mean of three replications.

Figures in parentheses represent arcsine transformed values.

Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Efficacy of Talc Based Bioformulations of Entomopathogenic Fungal Strains against *N. Lugens*

Application of talc based formulations of entomopathogenic fungal pathogens such as *Metarhizium,*(M1) strains were evaluated for their efficacy against *N. lugens* in rice plants under screen house conditions. The talc based bioformulation of *Metarhizium* (M1) strain and *Metarhizium* (commercial formulation Kothari Pvt Ltd) strain were found pathogenic to the *N. lugens*. The percent mortality varied from 60.0 to 83.0 per cent. Mortality per cent was 71.0 in M1 (NBAII) strain and 60.0 in *Metarhizium* commercial formulation (Kothari) while in chemical control it was 83.0. No mortality was observed in the control and all the treatments differed significantly (Table 4).

Feeding Preference of *N. Lugens* in Entomopathogenic Fungal Pathogens Treated Rice Plants

Feeding ability of *N. lugens* was significantly affected by entomopathogenic fungal pathogens. The results indicate that 36.95 mm^2 area of bromocresol green treated filter paper was stained with bluish white colour spots in *Metarhizium* (M1) (47.85 mm^2) strain treated plants. In chemical control, it was minimum (23.95 mm^2), while it was maximum in untreated control (173.75 mm^2) (Table 5).

Effect of Entomopathogenic Fungal Pathogens on Nymphal Emergence of BPH

The nymphal emergence of *N. lugens* was affected by entomopathogenic fungal pathogens. In *Metarhizium* (M1) and *Metarhizium* (Kothari) formulation treated plants, the nymphs emerged was 152.16 and 166.66 respectively. The least nymphal emergence was observed in chemical control (116.66).In untreated control, it was 254.16. The per cent reduction over control was 40.13 and 34.0. in *Metarhizium* (M1) and *Metarhizium* (Kothari) formulation, while in chemical control, the reduction was 54.10 (Table 5).

Table 5: Effect of Entomopathogenic Fungal Pathogens on Feeding Activity and Nymphal Emergence of BPH under Screen House Condition

S. No	Treatments	Feeding activity of BPH (mm^2)	No of BPH Nymphs Emerged	Per Cent Reduction Over Control
1.	*Metarhizium* strain (M1, NBAII) 0.5% foliar spray	36.95^b	152.16 $(12.36)^b$	40.13
2.	*Metarhizium* talc based (Commercial formulation of Kothari Pvt Ltd, for check) 0.5% foliar spray	47.85^c	166.66 $(12.93)^c$	34.43
3.	Chemical (Dimethoate, for check) 0.03% foliar spray	23.95^a	116.66 $(10.82)^a$	54.10
4.	Control inoculated	173.75^d	254.16 $(15.96)^d$	-

Values are mean of three replications.

Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Many strains of entomopathogenic fungal pathogens viz., *Beauveria bassiana* and *Metarhizium anisopliae* have been isolated from the soil of different crops by several workers (Callot *et al*. 1996; Sivasundaram *et al*. 2008; Storey *et al*.

1989; Shimazu *et al*., 2002) and tested against several insect pests of crops (Ambethgar 2003).Vinod kumar and Chowdhry, (2004) reported that LT_{50} value for *Metarhizium anisopliae* against tomato fruit borer *Helicoverpa armigera* ranged from 2.3 days to 8.0 days. Oliveira *et al*. (2002), reported *B. bassiana* isolates at 10^8 conidia ml-1 and the red mite *Oligonychus yothersi* (McGregor) recorded a variation in total mortality from 77 to 98%. The talc-based powder formulation of entomopathogenic fungal pathogen M1 was tried against brown plant hopper under glasshouse conditions. The results showed lowest damage (5.6%) compared to inoculated control (25.8%), which was supported by several authors (Padmaja and Kaur 2001; Padmanaban 1993; Aguda *et al.* 1987).

CONCLUSIONS

From these results, Metarhizium anisopliae was more effective against S. litura. This study give alternative method to chemical pesticides. The microbial biocontrol agents they are the natural enem ies devasting the pest population with no hazards effects on human health and the environment.

Entomopathogenic fungi have an important position among all the microbial biocontrol agents because of their route of pathogenicity, broad host range and ability to control insect pest

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